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UTILITY PATENT APPLICATION TRANSMITTAL (Large Entity)
Only for new nonprovisional applications under 37 C.F.R. 1.53(b)

Docket No.:
2874-B
Express Mail Label No.:
EL591095097US

JC917 U.S. PTO
09/705985

11/03/00

TO THE ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

METHOD OF INHIBITING OSTEOCLAST ACTIVITY

and invented by:

Dirk M. Anderson, residing at Seattle, Washington and Laurent J. Galibert residing at Seattle, Washington.

If a **CONTINUING APPLICATION**, check appropriate box and supply the requisite information:

☒ Continuation ☐ Divisional ☐ Continuation-in-part (CIP)

of prior application No.: PCT/US99/10588

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification including claims and abstract (13 pages total)
3. ☐ Drawing(s); Number of Sheets
4. ☒ Oath or Declaration
 - a. ☒ Newly executed
 - b. ☐ Copy from a prior application (37.C.F.R. 1.63(d)) (*for continuation/divisional application only*)
 - c. ☐ With Power of Attorney ☒ Without Power of Attorney
 - d. ☐ DELETION OF INVENTOR(S)

Signed statement attached deleting inventor(s) named in prior application, see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation by Reference (usable if Box 4b is checked)

The entire disclosure of the prior application from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Computer Program in Microfiche (Appendix)
7. ☒ Nucleotide and/or Amino Acid Sequence Submission
 - a. ☒ Paper copy
 - ☐ Pages - _____ of specification
 - ☒ Separately numbered pages 1 - 13
 - b. ☐ Computer Readable Copy
 - c. ☐ Statement Verifying Identical Paper and Computer Readable Copy
 - d. ☒ Statement under 37 C.F.R. 1.821(e) in lieu of Computer Readable Copy

Accompanying Application Parts

8. ☒ Assignment
- a. ☐ Executed original Assignment and Recordation Form enclosed
- b. ☐ Prior application is assigned of record to Immunex Corporation
(reel _____ frame_____)
9. ☒ 37 C.F.R. 3.73(B) Statement (*when there is an assignee*)
10. ☒ Preliminary Amendment
11. ☒ Acknowledgment postcard
12. ☒ Certificate of Mailing by Express Mail (Label No.: EL591095097US)
13. ☐ Certified Copy of Priority Document(s) (*if foreign priority is claimed*)
14. ☐ Additional Enclosures (*please identify below*):

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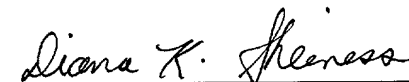
Fee Calculation and Transmittal

| CLAIMS AS FILED (after any Preliminary Amendment submitted herewith) | | | | | |
|--|---------|-----------|---------|-----------|----------|
| For | # Filed | # Allowed | # Extra | Rate | Fee |
| Total Claims | 24 | - 20 = | 4 | x \$18.00 | \$72.00 |
| Indep. Claims | 3 | - 3 = | 0 | x \$80.00 | \$0.00 |
| Multiple Dependent Claims (check if applicable) <input type="checkbox"/> | | | | | \$0.00 |
| BASIC FEE | | | | | \$710.00 |
| OTHER FEE (<i>specify purpose</i>) | | | | | \$0.00 |
| TOTAL FILING FEE | | | | | \$782.00 |

- ☒ The Commissioner is hereby authorized to charge and credit Deposit Account No. 09-0089 as described below. A copy of this sheet is enclosed.
- ☒ Charge the amount of \$782.00 as a filing fee.
- ☒ Credit any overpayment.
- ☒ Charge any additional fees required under 37 C.F.R. 1.16 and 1.17.

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Diana K. Sheiness
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Dated: November 3, 2000

| | | | |
|------------------------|--|-----------------|---------|
| In the Application of: | Dirk M. Anderson and Laurent J. Galibert | Docket No.: | 2874-B |
| Serial No: | --to be assigned-- | Group Art Unit: | Unknown |
| Filed: | November 3, 2000 | Examiner: | Unknown |
| For: | METHOD OF INHIBITING OSTEOCLAST ACTIVITY | | |

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Prior to examining the above-referenced patent application, please enter the following amendments into the application:

Between the Title and the Technical Field of the Invention, please insert the following paragraph:

- - This application is a continuation of PCT/US99/10588, filed May 13, 1999, which claims the benefit of priority from U.S. provisional applications 60/085,487, filed May 14, 1998, and 60/110,836, filed December 3, 1998, and is a continuation-in-part of United States patent application 08/966,139 (now U.S. 6,017,729), filed December 22, 1997, which claims the benefit of priority from U.S. provisional applications 60/064,671, filed October 14, 1997; 60/077,181, filed March 7, 1997; and 60/059,978, filed December 23, 1996. - -

In the Specification:

At page 3, line 31, please delete [a nucleotide]

At page 3, at line 7, between “NO:2.” and “Moreover” please insert:

-- Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between

196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:2, although other amino acids in the spacer region may be utilized as a C-terminus. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 214, and 197 of SEQ ID NO:5, although other amino acids in the spacer region may be utilized as a C-terminus. - -

At page 4, line 28, please delete [NO:6] and substitute therefor - - NO:5 - -

At page 7, between lines 5 and 6, please insert the following paragraph:

- - Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced induction of NF- κ B activity. NF- κ B is a transcription factor that is utilized extensively by cells of the immune system, and plays a role in the inflammatory response. Thus, inhibitors of RANK signalling will be useful in treating conditions in which signalling through RANK has given rise to negative consequences, for example, toxic or septic shock, or graft-versus-host reactions. They may also be useful in interfering with the role of NF- κ B in cellular transformation. Tumor cells are more responsive to radiation when their NF- κ B is blocked; thus, soluble RANK (or other antagonists of RANK signalling) will be useful as an adjunct therapy for disease characterized by neoplastic cells that express RANK. - -

In the claims:

1. A method of [regulating] inhibiting osteoclast [activity] generation, the method comprising [causing] administering to a patient in need thereof a therapeutic composition comprising a recombinant soluble RANK [to bind RANKL] polypeptide.

In Claim 2(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 2(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 2(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

5. (once amended) A method of ameliorating effects of excess bone loss, comprising administering a soluble RANK polypeptide composition to an individual at risk for excess bone loss[, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK].

6. (once amended) The method of claim 5, wherein the individual is at risk from or suffers from a condition selected from the group consisting of osteoporosis, [Pagett's] Paget's disease, [and] bone cancer, multiple myeloma, melanoma, breast cancer and cancers associated with hypercalcemia.

In Claim 7(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 7(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 7(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 10(a), line 3, please delete [62] and substitute therefor - - 2 - -

In Claim 10(b), line 2, please delete [NO:6] and substitute therefor - - NO:5 - -

In Claim 10(b), line 3, please delete [NO:6] and substitute therefor - - NO:5 - -

Please add the following new claims:

13. A method of ameliorating the effects of excess bone loss comprising administering to a patient in need thereof a therapeutic composition comprising a recombinant soluble RANK polypeptide, wherein said patient suffers from a condition selected from the group consisting of squamous cell carcinoma, lung cancer, prostate cancer, hematologic cancer, head and neck cancer and renal cancer.

14. The method of claim 13, wherein the soluble RANK polypeptide is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:2, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:5, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:5, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) a DNA capable of hybridizing to the DNA of (a) or (b) under stringent conditions, and that encodes a RANK polypeptide that binds RANKL; and

(d) a DNA molecule encoding a fragment of a protein encoded by a DNA of (a), (b) or (c), wherein said fragment binds RANKL.

15. The method of claim 14, wherein the soluble RANK polypeptide is at least about 80% identical in amino acid sequence to native RANK.

16. The method of claim 13, wherein the soluble RANK polypeptide further comprises one or more polypeptides selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues and a leucine zipper.

17. A method according to claim 4, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain comprising an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper comprising an amino acid sequence as shown in SEQ ID NO:6.

18. A method according to claim 9, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain comprising an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper comprising an amino acid sequence as shown in SEQ ID NO:6.

19. A method according to claim 12, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain having an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper having an amino acid sequence as shown in SEQ ID NO:6.

20. A method according to claim 16, wherein the further polypeptide is selected from the group consisting of an immunoglobulin Fc domain having an amino acid sequence as shown in SEQ ID NO:3 and a leucine zipper having an amino acid sequence as shown in SEQ ID NO:6.

21. A method according to claim 1, wherein the soluble RANK polypeptide comprises amino acids 34 through 196 of SEQ ID NO:2.

22. A method according to claim 5, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

23. A method according to claim 6, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

24. A method according to claim 13, wherein the soluble RANK comprises amino acids 34 through 196 of SEQ ID NO:2.

REMARKS

Claims 1-12 are pending in the application. Claims 1, 2, 5, 6, 7 and 10 have been amended as indicated above, and new Claims 13-24 have been added to the application. The specification has been amended to correct two inadvertent errors, and to add two excerpts derived from a prior application that was originally incorporated by reference into the present application.

The specification is amended at page 3, line 31 to delete the words "a nucleotide" in reference to SEQ ID NO:3. The sequence shown in SEQ ID NO:3 is clearly an amino acid sequence, thus it is self-evident that the words "a nucleotide" represent an inadvertent typing error. Accordingly, the deletion of these two words does not constitute the addition of new matter.

The specification is amended above by the addition of two excerpts taken from patent application 08/966,139 (now U.S. 6,017,729). Inserting these two excerpts into the application does not constitute the addition of new matter because USSN 08/966,139 is incorporated by reference into parent application PCT/US99/10588 (see the specification at page 2, line 35, to page 3, line 1). The first excerpt added herein is found in U.S. 6,027,729 at column 16, lines 14-28, and the second excerpt is found in U.S. 6,027,729, at column 17, line 66 to column 18, line 11, and at column 27, lines 4-13. The excerpts shown above are identical to the text at columns 16-18 of U.S. 6,027,729 except for the actual numbers of the SEQ ID NOS. In U.S. 6,027,729, human and mouse RANK proteins correspond, respectively, to SEQ ID NOS:6 and 14, whereas in the present application, human and mouse RANK proteins correspond, respectively, to SEQ ID NOS:2 and 5. Accordingly, the numbers "6" and "14" in the excerpts added herein were changed to "2" and "5." Since these substitutions ensure that the *same* amino acid sequences are referred to in both documents, they do not constitute the addition of new matter.

The specification is amended at page 4, line 28 to refer to "SEQ ID NO:5" rather than "SEQ ID NO:6." The text surrounding this recitation clearly refers to murine RANK. Taking the specification as a whole into account, it would be readily apparent to one skilled in the art that SEQ ID NO:5, not SEQ ID NO:6, is murine RANK. SEQ ID NO:6, for example, contains only 33 amino acids, thus obviously could not represent a full-length RANK protein (see the specification at page 1, lines 34-35, noting that human RANK has 616 amino acids). Furthermore, the specification at page 1, lines 9-11, refers to two U.S. patent applications that disclose the cloning of RANK and RANKL. One of these is 08/996,139 (now U.S. Patent No.6,017,729), which teaches that murine RANK is a protein having 625 amino acids (see U.S. 6,017,729 at column 26, lines 57-61 and surrounding text). SEQ ID NO:5 in the present application contains 625 amino acids. Moreover, the heading for SEQ ID NO:4 herein, which discloses the same protein as SEQ ID NO:5, identifies the sequence as "muRANK." Thus, this amendment merely corrects a self-evident error and does not constitute the addition of new matter.

Claims 1(a), 7(a) and 10(a) are amended above to refer to "SEQ ID NO:2" rather than "SEQ ID NO:62." The need for this correction is self-evident from the first two lines of these claims, which refer to "an amino acid sequence as set forth in SEQ ID NO:2;" it would be readily apparent to one skilled in the art that the second part of Claims

1(a), 7(a) and 10(a) are meant to refer back to the same sequence that was recited in the initial part of these claims. Furthermore, the sequence listing discloses only six sequences. Thus, it is self-evident that the reference to "NO:62" is an inadvertent error and that the correct sequence listing is SEQ ID NO:2. Accordingly, these amendments do not constitute the addition of new matter.

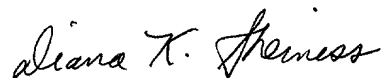
Claims 1(b), 7(b) and 10(b) are amended as shown above to substitute "SEQ ID NO:5" for "SEQ ID NO:6." Claims 1(b), 7(b) and 10(b) are meant to refer to murine RANK, which as explained above is SEQ ID NO:5, not SEQ ID NO:6. Thus, these amendments do not constitute the addition of new matter.

Claim 6 is amended to correct the inadvertent misspelling of "Paget's" and to delete an inadvertently included word that one skilled in the art would readily recognize as being superfluous. Thus, these amendments do not constitute the addition of new matter.

New Claim 13 is supported throughout the specification, for example, at page 7, line 13, to page 8, line 7. New Claim 14 is supported throughout the specification, for example, in originally filed Claims 2, 7 and 10; at page 3, lines 3-14; at page 4, lines 20-26; page 4, line 37 to page 5, line 5; and in the two excerpts added above by amendment to the specification. New Claim 15 is supported throughout the specification, for example, in originally filed Claims 3, 8 and 11 and at page 4, lines 26-31. New Claim 16 is supported throughout the specification, for example, in originally filed Claims 4, 9 and 12, and at page 3, line 22 to page 4, line 19. New Claims 17-20 are supported, for example, at page 3, lines 31-32, and at page 4, line 18. New Claims 21-24 are supported in the specification, for example, at page 3, lines 3-8. Thus, these new claims do not constitute the addition of new matter.

If the examiner has any questions about this application, he or she is urged to contact the undersigned at her direct dial telephone number given below.

Respectfully submitted,



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Registration No. 35,356
Telephone: (206) 470-4818

WO 99/58674

PCT/US99/10588

TITLE**METHOD OF INHIBITING OSTEOCLAST ACTIVITY****TECHNICAL FIELD OF THE INVENTION**

The present invention relates generally to the field of cytokine receptors, and more specifically to cytokine receptor/ligand pairs having osteoclast regulatory activity.

BACKGROUND OF THE INVENTION

RANK (Receptor Activator of NF- κ B) and its ligand (RANKL) are a recently-described receptor/ligand pair that play an important role in an immune response. The cloning of RANK and RANKL is described in USSN 08/996,139 and USSN 08/995,659, respectively. It has recently been found that RANKL binds to a protein referred to as osteoprotegerin (OPG), a member of the Tumor Necrosis Factor Receptor (TNFR) family. Yasuda et al. (*Proc. Natl. Acad. Sci.* 95:3597; 1998) expression cloned a ligand for OPG, which they referred to as osteoclastogenesis inhibitory factor. Their work was repeated by Lacey et al. (*Cell* 93:165; 1998). In both cases, the ligand they cloned turned out to be identical to RANKL.

In osteoclastogenesis, the interaction of an osteoblast or stromal cell with an osteoclast precursor leads to the differentiation of the precursor into an osteoclast. OPG was known to inhibit this differentiation. A model has been proposed in which RANKL on the osteoblast or stromal cell surface interacts with a specific receptor on an osteoclast progenitor surface, signaling a differentiation event. OPG effectively blocks the interaction of RANKL with a receptor on osteoclast progenitors *in vitro*, and has been shown to ameliorate the effects of ovariectomy on bone-loss in mice. However, OPG is also known to bind other ligands in the TNF family, which may have a deleterious effect on the activities of such ligands *in vivo*. Moreover, the presence of other ligands that bind OPG *in vivo* may require high dosages of OPG to be administered in order to have sufficient soluble OPG available to inhibit osteoclastogenesis.

Accordingly, there is a need in the art to identify soluble factors that specifically bind RANKL and inhibit the ability of RANKL to induce osteoclastogenesis without reacting with other ligands.

SUMMARY OF THE INVENTION

The present invention provides processes associated with the use of a novel receptor, referred to as RANK (for receptor activator of NF- κ B), that is a member of the TNF receptor superfamily. RANK is a Type I transmembrane protein having 616 amino acid residues, comprising an extracellular domain, transmembrane region and cytoplasmic domain. RANK interacts with various TNF Receptor Associated Factors (TRAFs);

triggering of RANK results in the upregulation of the transcription factor NF- κ B, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

5 Soluble forms of the receptor can be prepared and used to interfere with signal transduction through membrane-bound RANK. Inhibition of RANKL-mediated signal transduction will be useful in ameliorating the effects of osteoclastogenesis and osteoclast activity in disease conditions in which there is excess bone break down. Examples of such conditions include osteoporosis, Paget's disease, cancers that may metastasize to bone and induce bone breakdown (i.e., multiple myeloma, breast cancer, some
10 melanomas; see also Mundy, C. *Cancer Suppl.* 80:1546; 1997), and cancers that do not necessarily metastasize to bone, but result in hypercalcemia and bone loss (e.g. squamous cell carcinomas).

Soluble forms of RANK comprise the extracellular domain of RANK or a fragment thereof that binds RANKL. Fusion proteins of RANK may be made to allow
15 preparation of soluble RANK. Examples of such fusion proteins include a RANK/Fc fusion protein, a fusion protein of a zipper moiety (i.e., a leucine zipper), and various tags that are known in the art. Other antagonists of the interaction of RANK and RANKL (i.e., antibodies to RANKL, small molecules) will also be useful in the inventive methods. These and other aspects of the present invention will become evident upon
20 reference to the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified and was used to hybridize to colony blots generated
25 from a dendritic cell (DC) cDNA library containing full-length cDNAs. SEQ ID NO:1 shows the nucleotide and amino acid sequence of a predicted full-length protein.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about
30 four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the
35 immune and inflammatory response. The isolation of a DNA encoding RANK is described in USSN 08/996,139, filed December 22 1997, the disclosure of which is

incorporated by reference herein. USSN 08/996,139 describes several forms of RANK that are useful in the present invention.

Soluble RANK comprises the signal peptide and the extracellular domain (residues 1 to 213 of SEQ ID NO:2) or a fragment thereof. Alternatively, a different
5 signal peptide can be substituted for the native leader, beginning with residue 1 and continuing through a residue selected from the group consisting of amino acids 24 through 33 (inclusive) of SEQ ID NO:2. Moreover, fragments of the extracellular domain will also provide soluble forms of RANK.

Fragments can be prepared using known techniques to isolate a desired portion of
10 the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNFR family (of which RANK is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANK proteins within the scope of this invention include
15 covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conjugated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-
20 translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α -factor leader).

Protein fusions can comprise peptides added to facilitate purification or
identification of RANK proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as
25 that described by Hopp et al., *Bio/Technology* 6:1204 (1988; FLAGTM). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein.

Fusion proteins further comprise the amino acid sequence of a RANK linked to an
30 immunoglobulin Fc region. An exemplary Fc region is a human IgG₁ having a nucleotide an amino acid sequence set forth in SEQ ID NO:3. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to Fc γ RI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that Leu₍₂₃₄₎ and Leu₍₂₃₅₎ were critical to high affinity
35 binding of IgG₃ to Fc γ RI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG₁ Fc region to decrease the affinity of IgG₁

for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANK regions.

5 In another embodiment, RANK proteins further comprise an oligomerizing peptide such as a zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for multimerization of the proteins. The zipper domain comprises a
10 repetitive heptad repeat, with four or five leucine, isoleucine or valine residues interspersed with other amino acids. Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, *fos* and *jun*, also exhibit zipper domains, as does the gene product of the murine proto-oncogene, *c-myc* (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes *fos* and *jun* comprise zipper domains that preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). A preferred zipper moiety is that of SEQ ID NO:6 or a fragment thereof. This and other zippers are disclosed in US Patent 5,716,805.

20 Other embodiments of useful proteins include RANK polypeptides encoded by DNAs capable of hybridizing to the DNA of SEQ ID NO:1 under moderately stringent conditions (prewashing solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50°C, 5 X SSC, overnight) to the DNA sequences encoding RANK, or more preferably under stringent conditions (for example, hybridization in 6 X
25 SSC at 63°C overnight; washing in 3 X SSC at 55°C), and other sequences which are degenerate to those which encode the RANK. In one embodiment, RANK polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANK protein as set forth in SEQ ID NO:2 for human RANK and NO:6 for murine RANK. In a preferred embodiment, RANK polypeptides are at least about 80%
30 identical in amino acid sequence to the native form of RANK; most preferred polypeptides are those that are at least about 90% identical to native RANK.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). For
35 fragments derived from the RANK protein, the identity is calculated based on that portion of the RANK protein that is present in the fragment

The biological activity of RANK analogs or muteins can be determined by testing the ability of the analogs or muteins to bind RANKL, for example as described in the

Examples herein. Suitable assays include, for example, an enzyme immunoassay or a dot blot, and assays that employ cells expressing RANKL. Suitable assays also include, for example, inhibition assays, wherein soluble RANK is used to inhibit the interaction of RANKL with membrane-bound or solid-phase associated RANK (i.e., signal transduction assays). Such methods are well known in the art.

RANKL and RANK are important factors in osteoclastogenesis. RANK is expressed on osteoclasts and interacts with RANK ligand (RANKL) to mediate the formation of osteoclast-like (OCL) multinucleated cells. This was shown by treating mouse bone marrow preparations with M-CSF (CSF-1) and soluble RANKL for 7 days in culture. No additional osteoclastogenic hormones or factors were necessary for the generation of the multinucleated cells. Neither M-CSF nor RANKL alone led to the formation of OCL. The multinucleated cells expressed tartrate resistant acid phosphatase and were positive for [¹²⁵I]- calcitonin binding. The tyrosine kinase c-src was highly expressed in multinucleated OCL and a subset of mononuclear cells as demonstrated by immunofluorescence microscopy. (See Example 2).

Purification of Recombinant RANK

Purified RANK, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANK and homologs thereof. For example, a RANK expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANK protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing

zipper domain. Monoclonal antibodies against the RANK protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANK.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANK composition. Suitable methods include those analogous to the method disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Uses and Administration of RANK Compositions

The present invention provides methods of using therapeutic compositions comprising a protein and a suitable diluent and carrier. These methods involve the use of therapeutic compositions of RANK or soluble fragments of RANK for regulating an immune or inflammatory response. Further included within the present invention are methods for regulating osteoclast activity by administering therapeutic compositions of RANK or soluble RANK fragments to an individual in amounts sufficient to decrease excess bone resorption. Typically, the individual is inflicted with excess bone resorption and suffers from the effects of hypercalcemia, has symptoms of hypercalcemia, or is suffering a disease that involves excessive bone resorption. In addition to regulating osteoclast activity, the methods described herein are applicable to inhibiting osteoclast

activity, regulating osteoclast generation and inhibiting osteoclast generation in individuals inflicted with excess bone resorption. In connection with the methods described herein, the present invention contemplates the use of RANK in conjunction with soluble cytokine receptors or cytokines, or other osteoclast/osteoblast regulatory molecules.

In connection with the methods described herein, RANK ligand (RANKL) on osteoblasts or stromal cells is known to interact with RANK on osteoclast progenitor surfaces signaling an event that leads to the differentiation of osteoclast precursors into osteoclasts. (See Example 2 below.) Thus, RANK, and in particular soluble forms of RANK, is useful for the inhibition of the RANKL-mediated signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Soluble forms of RANK are also useful for the regulation and inhibition of osteoclast activity, e.g. bone resorption. By interfering with osteoclast differentiation, soluble forms of RANK are useful in the amelioration of the effects of osteoclastogenesis in disease conditions in which there is excess bone break down. Such disease conditions include Paget's disease, osteoporosis, and cancer. Many cancers metastasize to bone and induce bone breakdown by locally disrupting normal bone remodeling. Such cancers can be associated with enhanced numbers of osteoclasts and enhanced amount of osteoclastic bone resorption resulting in hypercalcemia. These cancers include, but are not limited to, breast cancer, multiple myeloma, melanomas, lung cancer, prostate, hematologic, head and neck, and renal. (See Guise et al. *Endocrine Reviews*, 19(1):18-54, 1998.) Soluble forms of RANK can be administered to such cancer patients to disrupt the osteoclast differentiation pathway and result in fewer numbers of osteoclast, less bone resorption, and relief from the negative effects of hypercalcemia.

Other cancers do not metastasize to bone, but are known to act systemically on bone to disrupt bone remodeling and result in hypercalcemia. (See Guise et al. *Endocrine Reviews*, 19(1):18-54, 1998.) In accordance with this invention, RANKL has been found on the surface of certain squamous cells that do not metastasize to bone but are associated with hypercalcemia. (See Example 3 below) Squamous cells that are associated with hypercalcemia also express M-CSF (CSF-1), a cytokine that, together with RANKL, stimulates the proliferation and differentiation of osteoclast precursors to osteoclasts. In accordance with the present invention, it has been discovered that M-CSF directly upregulates RANK on surfaces of osteoclast precursors. When squamous cells release excessive amounts of CSF-1, increased expression of RANK occurs on the surfaces of osteoclast precursors. Thus, there is a higher probability that RANK will interact with RANKL on osteoblasts or stromal cells to produce increased numbers of osteoclasts, resulting in an enhanced amount of bone break down and hypercalcemia.

In addition to the ameliorating the effects of cancers that metastasize to bone, the present invention provides methods for ameliorating the systemic effects, e.g. hypercalcemia, of cancers that are associated with excess osteoclast activity (e.g. squamous cell carcinomas). Such methods include administering soluble forms of RANK
5 in amounts sufficient to interfere with the RANK/RANKL signal transduction that leads to the differentiation of osteoclast precursors into osteoclasts. Fewer osteoclasts lead to reduced bone resorption and relief from the negative effects of hypercalcemia.

For therapeutic use, purified protein is administered to an individual, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example,
10 RANK protein compositions administered to regulate osteoclast function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANK, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages
15 and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrans, chelating agents such as EDTA, glutathione and other
20 stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being
25 treated, the desired response, the condition of the patient, and so forth.

Soluble forms of RANK and other RANK antagonists such as antagonistic monoclonal antibodies can be administered for the purpose of inhibiting RANK-induced osteoclastogenesis. It is desirable to inhibit osteoclastogenesis in various disease states in which excess bone loss occurs. Examples include osteoporosis, Pagett's disease, and
30 various cancers. Various animal models of these diseases are known in the art; accordingly, it is a matter of routine experimentation to determine optimal dosages and routes of administration of soluble RANK, first in an animal model and then in human clinical trials.

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

This example describes a plate binding assay useful in comparing the ability of various ligands to bind receptors. The assay is performed essentially as described in Smith et al., Virology 236:316 (1997). Briefly, 96-well microtiter plates are coated with an antibody to human Fc (i.e., polyclonal goat anti human Fc). Receptor/Fc fusion proteins are then added, and after incubation, the plates are washed. Serial dilutions of the ligands are then added. The ligands may be directly labeled (i.e., with ^{125}I), or a detecting reagent that is radioactively labeled may be used. After incubation, the plates are washed, specifically bound ligands are released, and the amount of ligand bound quantified.

Using this method, RANK/Fc and OPG/Fc were bound to 96-well plates. In an indirect method, a RANKL/zipper fusion is detected using a labeled antibody to the zipper moiety. It was found that human OPG/Fc binds mRANKL at 0.05 nM, and human RANK/Fc binds mRANKL at 0.1 nM. These values indicate similar binding affinities of OPG and RANK for RANKL, confirming the utility of RANK as an inhibitor of osteoclast activity in a manner similar to OPG.

EXAMPLE 2

The following describes the formation of osteoclast like cells from bone marrow cell cultures using a soluble RANKL in the form of soluble RANKL/leucine zipper fusion protein (RANKL LZ).

Using RANKL LZ at 1 $\mu\text{g}/\text{ml}$, osteoclasts were generated from murine bone marrow (BM) in the presence of CSF-1. These osteoclasts are formed by the fusion of macrophage-like cells and are characterized by their TRAP (tartrate-resistant acid phosphatase) positivity. No TRAP⁺ cells were seen in cultures containing CSF-1 alone or in cultures containing CSF-1 and TRAIL LZ (a control for the soluble RANKL LZ). Even though human and monkey bone marrow contains more contaminating fibroblasts than murine bone marrow, osteoclasts were generated from murine and monkey bone marrow with the combination of CSF-1 and soluble RANKL LZ. In a dose-response study using murine bone marrow and suboptimal amounts of CSF-1 (40ng/ml), the effects of soluble RANKL LZ plateaued at about 100ng/ml.

The effect of soluble RANKL LZ on proliferation of cells was studied in the same cultures using Alamar Blue. After 5 days, the proliferative response was lower in cultures containing CSF-1 and RANKL LZ than in those containing CSF-1 alone. The supports the observation that soluble RANKL LZ is inducing osteoclast differentiation. When CSF-1 and RANKL LZ are washed out of murine BM cultures at day 7 or 8, cells do not survive if they are recultured in medium or in RANKL LZ alone. In contrast, cells do

survive if recultured in CSF-1. When RANKL LZ was added to these cultures there was no added benefit. Thus, the combination of CSF-1 and RANKL are required for the generation of osteoclast. Additionally, once formed, CSF-1 is sufficient to maintain their survival in culture.

5 Finally, using human bone marrow, soluble anti-human RANK mAb and immobilized anti-human RANK mAb were compared to RANKL LZ for the generation of osteoclasts in the presence of CSF-1. Immobilized M331 and RANKL LZ were found to be equally effective for osteoclast generation while soluble M331 was superior to both immobilized antibody and RANKL LZ. This confirms that the osteoclast differentiating
10 activity of RANKL is mediated through RANK rather than via an alternative receptor.

Since osteoclasts cannot readily be harvested and analyzed by flow cytometry, ¹²⁵I-labeled calcitonin binding assays were used to identify osteoclasts (the calcitonin receptor is considered to be an osteoclast-specific marker). Osteoclasts generated from murine BM cultured with CSF-1 and RANKL LZ for 9 days showed binding of
15 radiolabeled calcitonin confirming their osteoclast identity.

EXAMPLE 3

In order to determine RANKL expression by either of two different squamous cell carcinomas, standard Western blot and RT-PCR studies were performed on MH-85 and
20 OKK cells. One of these carcinoma cells, the MH-85 cells, is associated with hypercalcemia.

The results confirmed that MH-85 and OKK squamous cells express RANKL. MH-85 cells, in addition to being linked with hypercalcemia in patients inflicted with this carcinoma, also express M-CSF (CSF-1). It was also determined that CSF-1 upregulates
25 RANK expression on osteoclast precursors. The enhanced amount of CSF-1 in MH-85 type squamous cell cancer patients can lead to an upregulation of RANK and increased RANK interaction with RANKL. Signals transduced by RANK and RANKL interaction result in increased numbers of mature osteoclasts and bone breakdown. Since soluble forms of RANK can inhibit the RANK/RANKL interaction, administering a soluble form
30 of RANK (e.g. the extracellular region of RANK fused to an Fc) to a squamous cell cancer patient provides relief from adverse effects of this cancer, including hypercalcemia.

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CLAIMS

We claim:

1. A method of regulating osteoclast activity, the method comprising causing a soluble RANK to bind RANKL.

2. The method of claim 1, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

3. The method of claim 2, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

4. The method of claim 3, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

5. A method of ameliorating effects of excess bone loss, comprising administering a soluble RANK polypeptide composition to an individual at risk for excess bone loss, and allowing the soluble RANK to bind RANKL and inhibit binding thereof to cells expressing RANK.

6. The method of claim 5, wherein the individual is at risk from or suffers from a condition selected from the group consisting of osteoporosis, Pagett's disease, and bone cancer, and cancers associated with hypercalcemia.

7. The method of claim 5, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

8. The method of claim 7, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

9. The method of claim 8, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAG™ tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

10. The method of claim 6, wherein the soluble RANK is encoded by a DNA selected from the group consisting of:

(a) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:2, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 33, inclusive, of SEQ ID NO:62, and a carboxy terminus selected from the group consisting an amino acid between amino acid 196 and amino acid 616, inclusive;

(b) a DNA encoding a protein having an amino acid sequence as set forth in SEQ ID NO:6, wherein the protein has an amino terminus selected from the group consisting of an amino acid between amino acid 1 and amino acid 30, inclusive, of SEQ ID NO:6, and a carboxy terminus selected from the group consisting of an amino acid between amino acid 197 and amino acid 625, inclusive;

(c) DNA molecules capable of hybridization to the DNA of (a) or (b) under stringent conditions, and which encode RANK polypeptides that bind RANKL; and

(d) DNA molecules encoding fragments of proteins encoded by the DNA of (a), (b) or (c), wherein the fragments of RANK polypeptides bind RANKL.

11. The method of claim 10, wherein the RANK is at least about 80% identical in amino acid sequence to native RANK

12. The method of claim 11, wherein the RANK further comprises a polypeptide selected from the group consisting of an immunoglobulin Fc domain, an immunoglobulin Fc mutein, a FLAGTM tag, a peptide comprising at least about 6 His residues, a leucine zipper, and combinations thereof.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Immunex Corporation
Anderson, Dirk M.
Galibert, Laurent
- (ii) TITLE OF INVENTION: METHOD OF INHIBITING OSTEOCLAST ACTIVITY
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
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 - (C) CITY: Seattle
 - (D) STATE: WA
 - (E) COUNTRY: USA
 - (F) ZIP: 98101
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM Compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #2.0
- (vi) CURRENT APPLICATION DATA:
 - (A) INT'L APPLICATION NUMBER: --to be assigned--
 - (B) FILING DATE: 13 May 1999
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Henry, Janis C.
 - (B) REGISTRATION NUMBER: 34,347
 - (C) REFERENCE/DOCKET NUMBER: 2874-WO
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (206)587-0430
 - (B) TELEFAX: (206)233-0644

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3136 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: HOMO SAPIENS
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS

(B) CLONE: FULL LENGTH RANK

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG      2886
TCGTTCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT      2946
CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAAACTC CAAGTTGCTG      3006
CAGCTTGCCA TTCTTCTTAT TCTAGAGGTC TCTCTGAAA AGATGGAGAA AATGAACAGG      3066
ACATGGGGCT CCTGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT      3126
TAAAAA      3136

```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 616 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
 1             5             10             15
Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
      20             25             30
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
      35             40             45
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
      50             55             60
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
      65             70             75             80
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
      85             90             95
Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
      100            105            110
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
      115            120            125
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
      130            135            140
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
      145            150            155            160
Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
      165            170            175

```

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
 180 185 190
 Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
 195 200 205
 Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
 210 215 220
 Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
 225 230 235 240
 Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
 245 250 255
 Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
 260 265 270
 Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
 275 280 285
 Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
 290 295 300
 Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
 305 310 315 320
 Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
 325 330 335
 Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
 340 345 350
 Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
 355 360 365
 Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
 370 375 380
 Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
 385 390 395 400
 Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
 405 410 415
 Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
 420 425 430
 His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
 435 440 445
 Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
 450 455 460
 Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
 465 470 475 480
 Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
 485 490 495
 Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
 500 505 510

Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
 515 520 525

Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
 530 535 540

Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
 545 550 555 560

Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
 565 570 575

Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
 580 585 590

Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
 595 600 605

Gln Glu Gln Gly Gly Ala Lys Ala
 610 615

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 232 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:

- (B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
 1 5 10 15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 20 25 30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 35 40 45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
 50 55 60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65 70 75 80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
 85 90 95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
 100 105 110

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
 115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
 130 135 140
 Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
 145 150 155 160
 His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
 165 170 175
 Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
 180 185 190
 Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
 195 200 205
 Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
 210 215 220
 Ser Leu Ser Leu Ser Pro Gly Lys
 225 230

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1878 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Murine
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: Murine Fetal Liver Epithelium
 - (B) CLONE: murANK
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | |
|---|-----|
| ATG GCC CCG CGC GCC CGG CGG CGC CGC CAG CTG CCC GCG CCG CTG CTG | 48 |
| Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu | |
| 1 5 10 15 | |
| GCG CTC TGC GTG CTG CTC GTT CCA CTG CAG GTG ACT CTC CAG GTC ACT | 96 |
| Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr | |
| 20 25 30 | |
| CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC | 144 |
| Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys | |
| 35 40 45 | |

| | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| AGC | AGA | TGC | GAA | CCA | GGA | AAG | TAC | CTG | TCC | TCT | AAG | TGC | ACT | CCT | ACC | 192 |
| Ser | Arg | Cys | Glu | Pro | Gly | Lys | Tyr | Leu | Ser | Ser | Lys | Cys | Thr | Pro | Thr | |
| | 50 | | | | | 55 | | | | | 60 | | | | | |
| TCC | GAC | AGT | GTG | TGT | CTG | CCC | TGT | GGC | CCC | GAT | GAG | TAC | TTG | GAC | ACC | 240 |
| Ser | Asp | Ser | Val | Cys | Leu | Pro | Cys | Gly | Pro | Asp | Glu | Tyr | Leu | Asp | Thr | |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 | |
| TGG | AAT | GAA | GAA | GAT | AAA | TGC | TTG | CTG | CAT | AAA | GTC | TGT | GAT | GCA | GGC | 288 |
| Trp | Asn | Glu | Glu | Asp | Lys | Cys | Leu | Leu | His | Lys | Val | Cys | Asp | Ala | Gly | |
| | | | | 85 | | | | | 90 | | | | | 95 | | |
| AAG | GCC | CTG | GTG | GCG | GTG | GAT | CCT | GGC | AAC | CAC | ACG | GCC | CCG | CGT | CGC | 336 |
| Lys | Ala | Leu | Val | Ala | Val | Asp | Pro | Gly | Asn | His | Thr | Ala | Pro | Arg | Arg | |
| | | | 100 | | | | | 105 | | | | | 110 | | | |
| TGT | GCT | TGC | ACG | GCT | GGC | TAC | CAC | TGG | AAC | TCA | GAC | TGC | GAG | TGC | TGC | 384 |
| Cys | Ala | Cys | Thr | Ala | Gly | Tyr | His | Trp | Asn | Ser | Asp | Cys | Glu | Cys | Cys | |
| | | 115 | | | | 120 | | | | | | 125 | | | | |
| CGC | AGG | AAC | ACG | GAG | TGT | GCA | CCT | GGC | TTC | GGA | GCT | CAG | CAT | CCC | TTG | 432 |
| Arg | Arg | Asn | Thr | Glu | Cys | Ala | Pro | Gly | Phe | Gly | Ala | Gln | His | Pro | Leu | |
| | 130 | | | | | 135 | | | | | 140 | | | | | |
| CAG | CTC | AAC | AAG | GAT | ACG | GTG | TGC | ACA | CCC | TGC | CTC | CTG | GGC | TTC | TTC | 480 |
| Gln | Leu | Asn | Lys | Asp | Thr | Val | Cys | Thr | Pro | Cys | Leu | Leu | Gly | Phe | Phe | |
| 145 | | | | | 150 | | | | | 155 | | | | | 160 | |
| TCA | GAT | GTC | TTT | TCG | TCC | ACA | GAC | AAA | TGC | AAA | CCT | TGG | ACC | AAC | TGC | 528 |
| Ser | Asp | Val | Phe | Ser | Ser | Thr | Asp | Lys | Cys | Lys | Pro | Trp | Thr | Asn | Cys | |
| | | | | 165 | | | | | 170 | | | | | 175 | | |
| ACC | CTC | CTT | GGA | AAG | CTA | GAA | GCA | CAC | CAG | GGG | ACA | ACG | GAA | TCA | GAT | 576 |
| Thr | Leu | Leu | Gly | Lys | Leu | Glu | Ala | His | Gln | Gly | Thr | Thr | Glu | Ser | Asp | |
| | | | 180 | | | | | 185 | | | | | 190 | | | |
| GTG | GTC | TGC | AGC | TCT | TCC | ATG | ACA | CTG | AGG | AGA | CCA | CCC | AAG | GAG | GCC | 624 |
| Val | Val | Cys | Ser | Ser | Ser | Met | Thr | Leu | Arg | Arg | Pro | Pro | Lys | Glu | Ala | |
| | | 195 | | | | 200 | | | | | | 205 | | | | |
| CAG | GCT | TAC | CTG | CCC | AGT | CTC | ATC | GTT | CTG | CTC | CTC | TTC | ATC | TCT | GTG | 672 |
| Gln | Ala | Tyr | Leu | Pro | Ser | Leu | Ile | Val | Leu | Leu | Leu | Phe | Ile | Ser | Val | |
| | 210 | | | | | 215 | | | | | 220 | | | | | |
| GTA | GTA | GTG | GCT | GCC | ATC | ATC | TTC | GGC | GTT | TAC | TAC | AGG | AAG | GGA | GGG | 720 |
| Val | Val | Val | Ala | Ala | Ile | Ile | Phe | Gly | Val | Tyr | Tyr | Arg | Lys | Gly | Gly | |
| 225 | | | | | 230 | | | | | 235 | | | | 240 | | |
| AAA | GCG | CTG | ACA | GCT | AAT | TTG | TGG | AAT | TGG | GTC | AAT | GAT | GCT | TGC | AGT | 768 |
| Lys | Ala | Leu | Thr | Ala | Asn | Leu | Trp | Asn | Trp | Val | Asn | Asp | Ala | Cys | Ser | |
| | | | | 245 | | | | | 250 | | | | | 255 | | |
| AGT | CTA | AGT | GGA | AAT | AAG | GAG | TCC | TCA | GGG | GAC | CGT | TGT | GCT | GGT | TCC | 816 |
| Ser | Leu | Ser | Gly | Asn | Lys | Glu | Ser | Ser | Gly | Asp | Arg | Cys | Ala | Gly | Ser | |
| | | | 260 | | | | | 265 | | | | | 270 | | | |
| CAC | TCG | GCA | ACC | TCC | AGT | CAG | CAA | GAA | GTG | TGT | GAA | GGT | ATC | TTA | CTA | 864 |
| His | Ser | Ala | Thr | Ser | Ser | Gln | Gln | Glu | Val | Cys | Glu | Gly | Ile | Leu | Leu | |
| | | 275 | | | | 280 | | | | | | 285 | | | | |
| ATG | ACT | CGG | GAG | GAG | AAG | ATG | GTT | CCA | GAA | GAC | GGT | GCT | GGA | GTC | TGT | 912 |
| Met | Thr | Arg | Glu | Glu | Lys | Met | Val | Pro | Glu | Asp | Gly | Ala | Gly | Val | Cys | |
| | 290 | | | | | 295 | | | | | 300 | | | | | |

GGG CCT GTG TGT GCG GCA GGT GGG CCC TGG GCA GAA GTC AGA GAT TCT 960
 Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
 305 310 315 320

AGG ACG TTC ACA CTG GTC AGC GAG GTT GAG ACG CAA GGA GAC CTC TCG 1008
 Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
 325 330 335

AGG AAG ATT CCC ACA GAG GAT GAG TAC ACG GAC CGG CCC TCG CAG CCT 1056
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
 340 345 350

TCG ACT GGT TCA CTG CTC CTA ATC CAG CAG GGA AGC AAA TCT ATA CCC 1104
 Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
 355 360 365

CCA TTC CAG GAG CCC CTG GAA GTG GGG GAG AAC GAC AGT TTA AGC CAG 1152
 Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
 370 375 380

TGT TTC ACC GGG ACT GAA AGC ACG GTG GAT TCT GAG GGC TGT GAC TTC 1200
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
 385 390 395 400

ACT GAG CCT CCG AGC AGA ACT GAC TCT ATG CCC GTG TCC CCT GAA AAG 1248
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
 405 410 415

CAC CTG ACA AAA GAA ATA GAA GGT GAC AGT TGC CTC CCC TGG GTG GTC 1296
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
 420 425 430

AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG 1344
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
 435 440 445

GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC 1392
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
 450 455 460

CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG 1440
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
 465 470 475 480

GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC 1488
 Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
 485 490 495

TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC 1536
 Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
 500 505 510

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG 1584
 Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
 515 520 525

AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG 1632
 Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
 530 535 540

GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT 1680
 Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
 545 550 555 560


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GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG 1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
565 570 575

CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG 1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
580 585 590

GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG 1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
595 600 605

GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA 1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
610 615 620

GAA TGA 1878
Glu
625

```

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 625 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
 1           5           10           15

Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
20           25           30

Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
35           40           45

Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
50           55           60

Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65           70           75           80

Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
85           90           95

Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
100          105          110

Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
115          120          125

Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
130          135          140

Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145          150          155          160

Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
165          170          175

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Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
 180 185 190
 Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
 195 200 205
 Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
 210 215 220
 Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly
 225 230 235 240
 Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
 245 250 255
 Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
 260 265 270
 His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
 275 280 285
 Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
 290 295 300
 Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
 305 310 315 320
 Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
 325 330 335
 Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
 340 345 350
 Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
 355 360 365
 Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
 370 375 380
 Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
 385 390 395 400
 Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
 405 410 415
 His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
 420 425 430
 Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
 435 440 445
 Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
 450 455 460
 Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
 465 470 475 480
 Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
 485 490 495
 Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
 500 505 510

Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
 515 520 525
 Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
 530 535 540
 Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
 545 550 555 560
 Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
 565 570 575
 Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
 580 585 590
 Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
 595 600 605
 Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
 610 615 620
 Glu
 625

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
 1 5 10 15
 Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
 20 25 30
 Arg

Immunex Corporation

Docket No.: 2874-B

DECLARATION OF INVENTORS

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF INHIBITING OSTEOCLAST ACTIVITY

the specification of which is filed herewith.

I hereby state that I have reviewed and understand the contents of said specification, including the claims that are presented in the Preliminary Amendment that is being submitted with the application. I acknowledge the duty to disclose information that is known to me and material to patentability of the subject claimed invention in accordance with 37 C.F.R. §1.56.


(X) I hereby claim the benefit under 35 U.S.C. §120 of the United States application(s) and PCT international application(s) designating the United States that are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) or PCT international application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

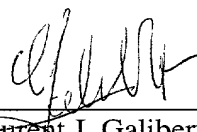
| <u>Application No.</u> | <u>Filed</u> |
|------------------------|---|
| PCT/US99/10588 | May 13, 1999 |
| USSN 08/966,139 | December 22, 1997 (now US Patent 6,017,729) |

(X) I hereby claim the benefit under 35 U.S.C. §119(e) of the United States provisional patent application(s) listed below:

| <u>Application No.</u> | <u>Filed</u> |
|------------------------|-------------------|
| 60/085,487 | May 14, 1998 |
| 60/110,836 | December 3, 1998 |
| 60/064,671 | October 14, 1997 |
| 60/077,181 | March 7, 1997 |
| 60/059,978 | December 23, 1996 |

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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